FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	ATTORNEY'S DOCKET NUMBER: BO 42260 U.S. APPLN. NO. (If known, see 37 CFR 1.5) 09/857796

INTERNATIONAL APPLICATION NO.:	INTERNATIONAL FILING DATE:	PRIORITY DATE CLAIMED:
PCT/NL99/00755	9 December 1999	9 December 1998

TITLE OF INVENTION: PREPARATION THAT CONTAINS OLIGOSACCHARIDES AND PROBIOTICS

APPLICANT(S) FOR DO/EO/US: Patricia DE JONG and Katrien VAN LAERE

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau. (see attached copy of PCT/IB/308)
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Item 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A **FIRST** preliminary amendment.
    [ ] A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:

    International Preliminary Examination (PCT/IPEA/409)
    International Search Report (PCT/ISA/210)
    Forms PCT/IB/304 and PCT/IB/308
    Abstract of the Disclosure on a separate sheet
    Application Data Sheet

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NO.
09/ 857796	PCT/NL99/00755	BO 42260

	CALCULATIONS PTO USE ONLY
17. [X] The following fees are submitted:	
BASIC *NATIONAL* FEE (37 CFR 1.492(a)(1)-(5)):	
Neither international preliminary examination fee (37 CFR1.482) nor international search fee (37 CFR1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... $ 1,000.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... $ 860.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... $ 710.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... $ 690.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... $ 100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00
Surcharge of $130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)).	$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	13 - 20 =	0	X $18.00	$
Independent claims	1 - 3 =	0	X $80.00	$
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$
		TOTAL OF ABOVE CALCULATIONS =		$ 860.00
Reduction of ½ for filing by small entity, if applicable. Applicant claims Small Entity Status under 37 CFR 1.27. +				$
		SUBTOTAL =		$ 860.00
Processing fee of $130 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR1.49(f)).				$
		TOTAL NATIONAL FEE =		$ 860.00
Fee for recording the enclosed assignment (37 CFR1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$ 40.00
		TOTAL FEES ENCLOSED =		$ 900.00
			Amount to be refunded:	
			charged:	

a. [X] A check in the amount of $ **900.00** to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. **25-0120** in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required by 37 CFR 1.16 and 1.17, or credit any overpayment to Deposit Account No. **25-0120**. A duplicate copy of this sheet is enclosed.

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June 11, 2001

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PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Patricia DE JONG et al. Box PCT

Serial No. (unknown) (PCT/NL99/00755) Application Branch

Filed herewith

PREPARATION THAT CONTAINS OLIGOSACCHARIDES AND PROBIOTICS

PRELIMINARY AMENDMENT

Commissioner for Patents

Washington, D.C. 20231

Sir:

Prior to the first Official Action and calculation of the filing fee, please substitute Claims 1-10 as originally filed, which appear on pages 10 and 11, with Claims 1-9 as filed in the Article 34 amendment of 27 November 2000. The pages containing Claims 1-9 are marked "AMENDED SHEET" and are attached hereto. Following the insertion of the Article 34 Claims 1-9, please amend these claims as follows:

IN THE CLAIMS:

Amend claim 2 as follows:

--2. (amended) Preparation according to Claim 1, wherein the oligosaccharides have a degree of polymerization of 2 to 20.--

Amend claim 3 as follows:

--3. (amended) Preparation according Claim 1, wherein the oligosaccharides have been obtained by the

hydrolysis of one or more polysaccharides, chosen from β-(arabino)galactans, β-(arabino)xylans, β-glucans, β-glucomannans, β-galactomannans, α-arabans, inulin and combinations thereof.--

Amend claim 5 as follows:

--5. (amended) Preparation according to Claim 1, wherein the oligosaccharides originate from the hydrolysis of one or more fibres, such as fibres originating from oats, wheat, potatoes, sugar beet, soya polysaccharides and the like.--

Amend claim 6 as follows:

--6. (amended) Preparation according to claim 1, wherein at least one of the bacterial strains is chosen from one or more strains of a *Lacto-bacillus* or a *Bifidobacterium* species and the yeast strain is a strain of a Saccharomyces species.--

Amend claim 7 as follows:

--7. (amended) Preparation according to claim 1 which also contains dead yeast cells.--

Amend claim 8 as follows:

--8. (amended) Preparation according claim 1, wherein the ratio between the one or more probiotics and the one or more non-digestible oligo-saccharides is in the range of 1 to 5 g oligosaccharides per 10^8 to 10^{11} cells of the probiotic.--

Amend claim 9 as follows:

--9. (amended) Preparation according to claim 1 which contains the probiotics in a concentration fo 10^7 to 10^{11} live cells per gram of total product.--

Add the following new claims:

--10. (new) Preparation according to Claim 1, wherein the oligosaccharides have a degree of polymerization of 2 to 10.

--11. (new) Preparation according to Claim 1, wherein the oligosaccharides have a degree of polymerization of 2 to 6.

--12. (new) Preparation according Claim 2, wherein the oligosaccharides have been obtained by the hydrolysis of one or more polysaccharides, chosen from β-(arabino)galactans, β-(arabino)xylans, β-glucans, β-glucomannans, β-galactomannans, α-arabans, inulin and combinations thereof.

--13. (new) Preparation according to Claim 2, wherein the oligosaccharides originate from the hydrolysis of one or more fibres, such as fibres originating from oats, wheat, potatoes, sugar beet, soya polysaccharide and the like.--

R E M A R K S

The above changes in the claims merely place this national phase application in the same condition as it was during Chapter II of the international phase, with the multiple dependencies being removed. Following entry of this

amendment by substitution of the pages, only Article 34 claims 1-9 remain pending in this application.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully submitted,

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June 11, 2001

"VERSION WITH MARKINGS TO SHOW CHANGES MADE

Amend claim 2 as follows:

--2. (amended) Preparation according to Claim 1, wherein the oligosaccharides have a degree of polymerization of 2 to 20~~, preferably of 2 to 10, more preferentially of 2 to 6~~.--

Amend claim 3 as follows:

--3. (amended) Preparation according Claim 1~~ or 2~~, wherein the oligosaccharides have been obtained by the hydrolysis of one or more polysaccharides, chosen from β-(arabino)galactans, β-(arabino)xylans, β-glucans, β-glucomannans, β-galactomannans, α-arabans, inulin and combinations thereof.--

Amend claim 5 as follows:

--5. (amended) Preparation according to Claim 1~~ or 2~~, wherein the oligosaccharides originate from the hydrolysis of one or more fibres, such as fibres originating from oats, wheat, potatoes, sugar beet, soya polysaccharides and the like.--

Amend claim 6 as follows:

--6. (amended) Preparation according to ~~one of the preceding~~ claim~~s~~ 1, wherein at least one of the bacterial strains is chosen from one or more strains of a *Lacto-bacillus* or a *Bifidobacterium* species and the yeast strain is a strain of a Saccharomyces species.--

Amend claim 7 as follows:

--7. (amended) Preparation according to ~~one of the preceding~~ claims 1 which also contains dead yeast cells.--

Amend claim 8 as follows:

--8. (amended) Preparation according ~~one of the preceding~~ claims 1, wherein the ratio between the one or more probiotics and the one or more non-digestible oligo-saccharides is in the range of 1 to 5 g oligosaccharides per 10^8 to 10^{11} cells of the probiotic.--

Amend claim 9 as follows:

--9. (amended) Preparation according to ~~one of the preceding~~ claims 1 which contains the probiotics in a concentration fo 10^7 to 10^{11} live cells per gram of total product.--

27. 11. 2000

New claims

(42)

1. Preparation having a health promoting action, in particular for the prevention and/or treatment of disorders of the digestive tract, which contains probiotics comprising at least one bacterial strain and at least one yeast strain and one or more non-digestible oligosaccharides.

2. Preparation according to Claim 1, wherein the oligosaccharides have a degree of polymerisation of 2 to 20, preferably of 2 to 10, more preferentially of 2 to 6.

3. Preparation according to Claim 1 or 2, wherein the oligosaccharides have been obtained by the hydrolysis of one or more polysaccharides, chosen from β-(arabino)galactans, β-(arabino)xylans, β-glucans, β-glucomannans, β-galactomannans, α-arabans, inulin and combinations thereof.

4. Preparation according to Claim 3, wherein the polysaccharides are chosen from β-(arabino)galactans, β-mannans, xylans and combinations thereof.

5. Preparation according to Claim 1 or 2, wherein the oligosaccharides originate from the hydrolysis of one or more fibres, such as fibres originating from oats, wheat, potatoes, sugar beet, soya polysaccharides and the like.

6. Preparation according to one of the preceding claims, wherein at least one of the bacterial strains is chosen from one or more strains of a *Lactobacillus* or a *Bifidobacterium* species and the yeast strain is a strain of a *Saccharomyces* species.

7. Preparation according to one of the preceding claims which also contains dead yeast cells.

8. Preparation according to one of the preceding claims, wherein the ratio between the one or more probiotics and the one or more non-digestible oligosaccharides is in the range of 1 to 5 g oligosaccharides per 10^8 to 10^{11} cells of the probiotic.

9. Preparation according to one of the preceding claims which contains the probiotics in a concentration of 10^7 to 10^{11} live cells per gram of total product.

Preparation that contains oligosaccharides and probiotics

The present invention relates to a preparation which has a health-promoting action, in particular for the prevention and/or treatment of disorders of the digestive tract, more particularly of the intestines.

The application relates in particular to a preparation of this type which contains probiotics and non-digestible oligosaccharides.

It is known that certain microorganisms have both a prophylactic and a therapeutic effect on intestinal diseases, such as intestinal infections. When these microorganisms are administered to humans or animals they will compete with pathogenic bacteria for nutrients and/or adhesion sites on the intestinal wall, as a result of which the number of pathogenic bacteria will decrease and infections are prevented or reduced. Such microorganisms are generally designated by the term "probiotics".

If these microorganisms are to have an optimum action they must reach the intestines alive. A further beneficial effect of the administration of live microorganisms to the intestines is, for example, that they are able to ferment the oligosaccharides present in the intestines, whereby, for example, fatty acids with short chains are formed.

In addition it is advantageous on economic grounds if as many microorganisms as possible reach the intestines alive. With the customary preparations which contain probiotics the percentage of microorganisms that reaches the intestines alive is frequently low.

It is therefore an object of the present invention to provide a preparation that contains such probiotics with which a high percentage of the microorganisms administered reaches the intestines alive.

It is also an object of the present invention to provide a preparation that can be used for the treatment of disorders of the intestines and/or that can be used for prophylactic treatment of the intestines.

The present invention now relates to a preparation having a health-promoting action, in particular for the prevention and/or treatment of disorders of the digestive tract, more particularly of the intestines, which contains probiotics and non-digestible oligosaccharides.

Without wishing to be tied to any theory, it is assumed that the oligosaccharides form a substrate for the probiotics, as a result of which the likelihood that said

microorganisms reach the intestines alive increases and as a result of which the likelihood that they are able, in combination with the oligosaccharides present in the intestines, to exert their beneficial action increases. The oligosaccharides could therefore also be designated as "prebiotics".

The probiotics and non-digestible oligosaccharides are present in the preparation in a ratio of 1 to 5 g oligosaccharides per 10^8 to 10^{11} cells of the probiotic.

The oligosaccharides used are chosen from the so-called "non-digestible oligosaccharides", that is to say oligosaccharides which are not absorbed by the human or animal body. These oligosaccharides as a rule have a degree of polymerisation of 2 to 20. This implies that the oligosaccharides consist of 2 to 20 monosaccharide units. In the case of a degree of polymerisation of less than 2, that is to say in the case of the monosaccharide, the preparation is not effective since such monosaccharides are absorbed by the human or animal body. At a degree of polymerisation of greater than 20 the oligosaccharides lose their beneficial action.

Preferably the oligosaccharides have a degree of polymerisation of 2 to 10, more preferentially of 2 to 6, more particularly 3 to 10 and more preferentially 3 to 6.

Where reference is made in the present application to oligosaccharides this term is used to refer both to oligosaccharides having one specific chain length and to mixtures of oligosaccharides having different chain lengths. However, a mixture of oligosaccharides having different chain lengths is preferred.

Furthermore, the oligosaccharides usually do not consist entirely of disaccharides. The disaccharide content is usually less than 90 % and sometimes less than 60 %.

The average degree of polymerisation is as a rule more than 2.1, usually more than 2.5.

The oligosaccharides used in the invention are, as a rule, furthermore so chosen that they are at least 20 % usable as substrate for the probiotic microorganisms present in the preparation, as determined by high performance anion exchange chromatography.

Examples of suitable oligosaccharides are transgalacto-oligosaccharides (TOS), fructo-oligosaccharides (FOS) and combinations thereof.

It is particularly advantageous if the oligosaccharides are added to the preparation in the form of a hydrolysis product of one or more polysaccharides, for example chosen from β-(arabino)galactans, β-(arabino)xylans, β-glucans, β-glucomannans, β-galactomannans, α-arabans, inulin and combinations thereof. In addition to the oligosaccharides, such a

hydrolysis product can also contains yet further components, such as monosaccharides and saccharides having a degree of polymerisation higher than 20. However, the hydrolysis product must contain at least 50 % non-digestible oligosaccharides, preferably at least 70 %.

The polysaccharides which are preferably hydrolysed are β-(arabino)galactans, β-mannans, and xylans.

It is also possible to use a hydrolysis product of one or more fibres which are mainly made up of the abovementioned oligosaccharides, such as fibres from oats, wheat, potatoes, sugar beet, soya polysaccharides and the like.

The hydrolysis of the polysaccharide(s) and/or the fibre(s) can be carried out in a manner known per se, for example by the use of suitable enzymes.

The concentration of oligosaccharides in the preparation is such that 0.5 to 20 gram per day can be administered. If desired, this administration can be spread over the day, as long as the total quantity of the oligosaccharides administered remains in the range described above. In general the preparation will be administered 2 to 4 times per day.

In general the oligosaccharides will make up 5 wt.% to 50 wt.% of the total preparation.

The probiotics suitable for the present invention are generally known. They comprise, in general, one or more bacterial strains suitable for use in food preparations, such as lactic acid bacteria suitable for use in food preparations, or one or more yeast strains suitable for use in food preparations, or a combination thereof. These probiotics will usually have GRAS ("Generally Recognised As Safe") status.

Suitable bacterial strains are, for example, chosen from those which are described in European Application 97202900.3 in the name of the Applicant. Other possible probiotics are the Pediococci, Propionibacteria or *Leuconostoc* species. The *Lactobacillus*, and *Bifidobacterium* genera and combinations thereof are to be preferred.

The *Bifidobacterium* strain used can be any strain which is suitable for, and preferably is also approved for, administration to humans and animals, such as *Bifidobacterium, bifidum, Bifidobacterium breve, Bifidobacterium lactis* or *Bifidobacterium longum,* or a combination thereof.

The *Lactobacillus* strain is preferably so chosen that this produces mainly, preferably exclusively, dextrorotatary (L+) lactate. What is meant by this is that the lactate produced is less than 5 %, preferably less than 2 %, laevorotatory lactate. It is, of course, possible

that the microorganism produces other metabolites in addition to lactate and the beneficial action of the microorganism can (also) be based on the formation of these further metabolites.

Examples of these are *Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus (para)casei, Lactobacillus fermentum, Lactobacillus plantarum* and *Lactobacillus rhamnosus*, and combinations thereof.

The yeast strain used in the invention can be any strain which is suitable for, and preferably is also approved for, administration to humans and animals, such as of the genus *Saccharomyces*. Examples of suitable *Saccharomyces* species are *Saccharomyces cerevisiae* and *Saccharomyces boulardii*.

The yeast in the preparation can be alive or dead. Both dead and live yeast contains a high content of mannoproteins, which are able to prevent the adhesion of bacteria to the intestinal wall to a large extent. Administration of dead yeast offers advantages in the case of people who are suffering from inflammatory intestinal diseases.

If the yeast is used in dead form, at least one other live microorganism must be used. Said live organism can once again be chosen from both the *Lactobacillus, Bifidobacterium*, Pediococci, Propionibacteria and *Leuconostoc* strains, but can also be live *Saccharomyces*.

It is possible to use both a single microorganism and a mixture of microorganisms.

The total concentration of the probiotics is 10^6 to 10^{12}, preferably 10^9 to 10^{10}, live cells per gram of total product. If a combination of microorganisms is used, the minimum concentration of each of the microorganisms must be such that there can still be said to be live organisms, that is to say at least approximately 10 per gram of product. The total concentration of microorganisms must always be within the above specified range of 10^6 to 10^{12} live cells per gram of total product.

If dead *Saccharomyces cerevisae* is also used, this is administered in a quantity of 0.5 to 5 g per day.

The combination of *Lactobacillus rhamnosus* with transgalacto-oligosaccharide or hydrolysis products of (potato) galactan is found to be a particularly suitable combination of oligosaccharide and probiotic.

The suitability of a specific oligosaccharide for a specific probiotic can be determined by determining the capacity of the microorganism concerned to ferment said oligosaccharide or said oligosaccharide fraction. A specific method for this is given below

with the examples.

The administration forms of the preparations according to the invention are as a rule analogous to those which have been described in European Application 97202900.3 in the name of the Applicant, the contents of which are incorporated herein by reference. In this context it must be pointed out that according to the present invention also only one probiotic microorganism (including a yeast) can be present and that in addition to the one or more probiotic microorganisms one or more oligosaccharides can also be incorporated, in the quantities specified above.

The preparations according to the present invention can, among others, be administered in the form of a nutritional supplement, total nutrition and clinical nutrition. Reference is likewise made to European Application 97202900.3 for the specific additives which are added to such foods and the preparation and applications of such foods.

The probiotics are preferably added to the preparation in (freeze-)dried form. It is also possible to produce liquid preparations, but these must be stored cool. Furthermore, one or more of the microorganisms can be used in encapsulated form, for example in order to improve the shelf life.

If the preparation according to the invention is used as a nutritional supplement, it can be administered as such, can be mixed with a suitable drinkable liquid, such as water, yoghurt, milk or fruit juice, or can be mixed with solid or liquid food. In this context the nutritional supplement can be in the form of tablets, capsules, powders, sachets, pastilles, sweets, bars and corresponding administration forms, usually in the form of a unit dose.

A supplement according to the invention can, for example, have the following composition:

- probiotics: 10 - 40 wt.%
- oligosaccharides: 40 - 80 wt.%
- further additives: 0 - 40 wt.%,

to a total of 100 wt.%.

The preparation can also be in the form of a food preparation that is suitable for direct consumption, such as total or clinical nutrition. This can be either oral nutrition or nutrition for administration via a tube or catheter.

Such foods can be in solid form or liquid/drinkable form and can contain all customary additives for (total and/or clinical) nutrition, including proteins, vitamins, minerals, trace elements and the like.

A total nutrition according to the invention can, for example, have the following composition:

- probiotics: 0.1 - 10 wt.%
- oligosaccharides: 1 - 20 wt.%
- further additives: 75 - 95 wt.%

to a total of 100 wt.%.

According to a particular embodiment, the preparation according to the invention is in the form of a (supplement for a) baby food or a nutritional supplement for babies.

The preparations described above can be used for the same applications as those described in European Application 97202900.3 in the name of the Applicant, in particular in the treatment of disorders of the intestines, such as diarrhoea, such as can arise when travelling or after treatment with antibiotics, or which results from food poisoning. Another application is in the treatment of inflammatory bowel diseases (IBD), such as colitis ulcerosa and Crohn's disease. The preparations according to the invention are also suitable for patients who have a food allergy, such as an allergy to cow's milk or to gluten.

The probiotics and non-digestible oligosaccharides can also be used in baby food to prevent or treat intestinal problems.

The invention will now be explained with the aid of the following non-limiting examples.

Examples

To determine suitable combinations of oligosaccharides and probiotics the microorganisms listed below were tested to determine their capacity to ferment structurally different oligosaccharide fractions. Strains pre-cultured in liquid medium based on thioglycolate (Oxoid, Unipath Ltd, Basingstoke, Hampshire, UK) were subjected to sub-culture in thioglycolate to which 0.5 % (m/V) oligosaccharides were added. The sugar-free thioglycolate medium and the oligosaccharide solutions were sterilised separately for 15 minutes at 121 °C.

Following anaerobic incubation for 48 hours at 37 °C the pH was measured with the aid of a micro-pH meter (Sentron, Roden, The Netherlands). The changes in the residual oligosaccharide content and the formation of reaction products were determined using HPAEC (high performance anionic exchange chromatography). For HPAEC analysis the cultures were centrifuged and the supernatant liquor was diluted 10-fold with H_2O and

boiled for 5 minutes to stop the enzymatic activity. The purity of the strains was checked before and after fermentation.

The HPAEC system consisted of a Dionex Bio-LC GPM-II quaternary gradient module (Dionex Corporation, Sunnyval, CA, USA) equipped with a Dionex Carbopac PA-100 column (4 * 250 mm) in combination with a Carbopac PA-100 guard column (3 * 25 mm). The samples (20 µl) were injected using a Spectra Physics SP8880 autosampler. The oligomers were analysed using a gradient of sodium acetate in 100 mmol.l^{-1} NaOH.

The results of these experiments are given below in the table for a number of combinations of probiotics and substrates, where:

++ indicates complete fermentation

\+ indicates partial fermentation

\- indicates no or very limited fermentation

Bacteria	Substrate			
	Hydrolysis product of:			
	Arabino-galactans	Arabans	Arabinoxylans	Fructans
Bi. Breve	++	+	-	++
Bi. Longum	++	++	+	++
Bi. adolescentis	++	+	++	++
L. acidophilus	++	-	-	+
L. fermentum	++	-	-	+
K. pnuemoniae	++	-	-	+
C. perfingens	-	-	-	++

A few examples of preparations according to the present invention are given below.

Example I: Supplement

A suspension of *Lactobacillus rhamnosus* ATCC 7469 (Lb) was freeze-dried, a powder being obtained which contained at least 10^9 viable cells per gram powder. Transgalacto-oligosaccharides (TOS), obtained from lactose (Borculo Whey Products), were dissolved in water at 40 °C to a solids content of 25 % and this solution was spray-dried. The two powders were mixed in a TOS/Lb ratio of 4/1 until a homogeneous product was obtained. Sachets were filled with 2 - 5 g of this mixture, depending on the dosage

regime (5 g for one sachet per day; 3 g for two sachets per day). The contents of one sachet can, for example, be taken mixed in a glass of orange juice or milk.

Example II: Synbiotic bar

A 23 g bar was prepared from 4.0 g oat flakes, 4.0 g wheat flakes, 3.0 g puffed rice, 1.0 g crushed hazelnuts, 0.25 g honey, 3.0 g raisins, 1.5 g maltodextrin, 1.0 g freeze-dried *Lb rhamnosus*, 0.5 g baker's yeast (*Saccharomyces cerevisiae*; Gist Brocades) and 5.0 g transgalacto-oligosaccharides.

Example III: Method for hydrolysing vegetable fibres

A 20 % suspension of fibres, for example from wheat, potatoes, oats, soya polysaccharides, carob gum or sugar beet, in water was prepared. These sources of fibre are commercially available. The suspension was heated to a temperature of between 20 and 50 °C (preferably 35 - 45 °C), after which enzymes were added in a quantity of one part enzyme per 5 - 500 parts (m/m) substrate. The choice of the type of enzyme is dependent on the type of polysaccharide. Examples of suitable enzymes are Novoferm Pectinex Ultra s.p.-L, Pentopan and Ultra.s.p. (NOVO).

After 0.5 - 4 hours the reaction was terminated by heating, after which the solution thus obtained can be used as the oligosaccharide fraction in the preparations according to the invention, optionally after further filtration/purification or after concentrating.

Example IV: Synbiotic mixture for mixing with a complete enteral clinical nutrition

A mother batch of a powder mixture was prepared in accordance with the method of Example I. The powder consisted of 20 % hydrolysed wheat arabinoxylans, 20 % hydrolysed potato arabinogalactans, 20 % hydrolysed carob gum, 20 % hydrolysed sugar beet fibre (arabans), 15 % hydrolysed oat fibres (glucans) and 5 % *Bifidobacterium longum*. 5 g of the powder mixture is placed in a sachet. The contents of this sachet can be added to a standard enteral clinical nutrition a maximum of 30 min before use.

Example V: Synbiotic powder mixture for fortifying baby food

A synbiotic mixture was prepared in accordance with the method of Example I. The composition contains 10 % baker's yeast (Gist Brocades), 40 % mannoproteins, obtained from yeast, 25 % inulin and 25 % raffinose.

Example VI: Sweet that contains a synbiotic mixture

A 2 g sweet was prepared starting from 58 % digestible carbohydrates (glucose syrup), 35 % TOS, 4 % *Lactobacillus rhamnosus* ATCC 7469, and 2 % flavourings and colourants.

New claims

(42)

1. Preparation having a health promoting action, in particular for the prevention and/or treatment of disorders of the digestive tract, which contains probiotics comprising at least one bacterial strain and at least one yeast strain and one or more non-digestible oligosaccharides.

2. Preparation according to Claim 1, wherein the oligosaccharides have a degree of polymerisation of 2 to 20, preferably of 2 to 10, more preferentially of 2 to 6.

3. Preparation according to Claim 1 or 2, wherein the oligosaccharides have been obtained by the hydrolysis of one or more polysaccharides, chosen from β-(arabino)galactans, β-(arabino)xylans, β-glucans, β-glucomannans, β-galactomannans, α-arabans, inulin and combinations thereof.

4. Preparation according to Claim 3, wherein the polysaccharides are chosen from β-(arabino)galactans, β-mannans, xylans and combinations thereof.

5. Preparation according to Claim 1 or 2, wherein the oligosaccharides originate from the hydrolysis of one or more fibres, such as fibres originating from oats, wheat, potatoes, sugar beet, soya polysaccharides and the like.

6. Preparation according to one of the preceding claims, wherein at least one of the bacterial strains is chosen from one or more strains of a *Lactobacillus* or a *Bifidobacterium* species and the yeast strain is a strain of a *Saccharomyces* species.

7. Preparation according to one of the preceding claims which also contains dead yeast cells.

8. Preparation according to one of the preceding claims, wherein the ratio between the one or more probiotics and the one or more non-digestible oligosaccharides is in the range of 1 to 5 g oligosaccharides per 10^8 to 10^{11} cells of the probiotic.

9. Preparation according to one of the preceding claims which contains the probiotics in a concentration of 10^7 to 10^{11} live cells per gram of total product.

ABSTRACT OF THE DISCLOSURE

A preparation having a health-promoting action for the prevention and/or treatment of disorders of the digestive tract,contains one or more probiotics and one or more non-digestible oligosaccharides. The probiotics are preferably chosen from bacterial strains such as a strain of a *Lactobacillus* or a *Bifido bacterium* species and from yeast strains such as a strain of a *Saccharomyces* species.

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL DESIGN, NATIONAL STAGE OF PCT OR CIP APPLICATION)

As a below named inventor, I hereby declare that

My residence, post office address and citizenship are as stated below next to my name, I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Preparation that contains oligosaccharides and probiotics

the specification of which: (complete (a), (b) or (c) for type of application)

REGULAR OR DESIGN APPLICATION

a. [ ] is attached hereto.

b. [ ] was filed on as Application
Serial No. and was amended on
(if applicable)

PCT FILED APPLICATION ENTERING NATIONAL STAGE

c. [x] was described and claimed in International application No. PCT/NL99/00755
filed on 9 December 1999
and as amended on (if any)

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, paragraph 1.56(a).

In compliance with this duty there is attached an information disclosure statement 37 CFR 1.97

PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35. United States Code paragraph 119 of any foreign application (s) for patent of inventor's certificate listed below and have also identified below any foreign application for patent of inventor's certificate having a filing date before that of the application on which priority is claimed.

(complete (d) or (e))

d. [ ] no such applications have been filed
e. [X] such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION

Country	Application Number	Date of filing (day, month, year)	Date of Issue (day, month, year)	Priority claimed
The Netherlands	1010770	9 December 1998		Yes

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION

_____

_____

CONTINUATION-IN-PART

(Complete this part only if this is a continuation-in-part application)

I hereby declare claim the benefit under Title 35, United States code, paragraph 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claim of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, paragraph 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, paragraph 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.) (Filing date) (Status) (patented, pending, abandoned)

(Application Serial No.) (Filing date) (Status) (patented, pending, abandoned)

POWER OF ATTORNEY

As a named inventor, I hereby appoint the following attorney(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Robert J. PATCH, Reg. No. 17,355, Andrew J. PATCH, Reg. No. 32,925, Robert F. HARGEST, Reg. No. 25,590, Benoît CASTEL, Reg. No. 35,041, Eric Jensen, Reg. No. 37,855, and Thomas W. PERKINS, Reg. No. 33,027 and Roland E. Long, Jr. Reg. No. 41,949 c/o YOUNG & THOMPSON, Second Floor, 745 South 23rd Street, Arlington, Virginia 22202.

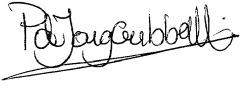
Address all telephone calls to Young & Thompson at 703/521-2297.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

Full name of sole or first inventor: **DE JONG, Patricia**

Inventor's signature



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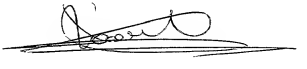
2-00

Full name of second inventor: **VAN LAERE, Katrien**

Inventor's signature



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